

Behavior of female *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in a mono-layer of wheat treated with diatomaceous earth[☆]

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Accepted 5 July 2006

Abstract

Adult female lesser grain borers (*Rhyzopertha dominica* [F.]) were observed in a mono-layer of wheat sandwiched between two layers of glass to determine if movement patterns and survival rates differed in wheat that was admixed with diatomaceous earth (DE) compared with untreated wheat. Observations were also made to determine if responses to DE differed depending on the commercial formulation of DE tested at the labeled rates. Mortality was higher in the DE treatments than in the untreated controls, and also varied according to the DE formulation. In wheat treated with 1000 ppm Dryacide[®], 400 ppm Protect-It[®], and 500 ppm Insecto[™] (labeled rate for the individual products), mean percentage mortality was 100 ± 0 , 71.4 ± 10.1 , and 57.1 ± 11.1 , respectively. Although total distance traveled and the number of turns taken by the beetles was lower in the Dryacide treatment compared to the untreated controls, the movement patterns were not significantly different among the three DE treatments. Observed differences in mortality are likely related to DE products or amount applied rather than to differences in DE exposure resulting from movement behavior.

Published by Elsevier Ltd.

Keywords: *Rhyzopertha dominica*; Diatomaceous earth; Wheat; Movement

1. Introduction

The lesser grain borer, *Rhyzopertha dominica* (F.), is a major pest of stored commodities worldwide (Potter, 1935). *Rhyzopertha dominica* generally infests stored wheat during the summer, and populations tend to increase as the weather gets warmer (Dowdy, 1994). The eggs are laid externally to the grain, and the first instar larva bores its way into the kernel and develops there until reaching the adult stage (Potter, 1935). This life history makes *R. dominica* a primary pest and one that is difficult to manage.

The current trend in stored-product pest management is to use reduced-risk or low-toxicity insecticides as a

replacement for conventional organophosphates. Several problems have emerged with the use of conventional chemical control methods. These include resistance by many species of stored-product pests, including *R. dominica* (Subramanyam and Hagstrum, 1996). Also, consumers generally prefer reduced pesticide residues on all agricultural products, including raw stored commodities (Arthur, 1996).

Diatomaceous earth (DE), an inert dust, is considered to be an alternative to conventional control methods. It is composed of fossilized cell walls of diatoms containing silica extracted by the living cells from their fluid environment. When the diatoms die, their cell walls collect at the bottom of oceans, lakes, and rivers (Quarles and Winn, 1996). The end product abrades the insect cuticle, absorbs lipids from the epicuticle, and causes death by desiccation and water loss (Golob, 1997; Korunic, 1998; Subramanyam and Roesli, 2000). There are several formulations of DE available commercially in the United States (USA) and throughout the world.

[☆]This paper reports the results of research only. Mention of a proprietary product or insecticide does not constitute a recommendation or endorsement by Kansas State University or the US Department of Agriculture.

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Rhyzopertha dominica usually migrates into bulk stored grain bins through air vents or cracks and crevices in the headspace (Hagstrum et al., 1994; Hagstrum, 2000). After entering a bin, *R. dominica* alights on the grain surface and then gradually moves downwards through the grain mass (Sharangapani and Pingale, 1957; Surtees, 1965; Keever, 1983; Hagstrum et al., 1994; Vela-Coiffier et al., 1997; Hagstrum, 2001). These studies, however, used large-scale arenas, measured only population movement patterns, and used sampling methods that were destructive. Moreover, the movement behavior of *R. dominica* in response to DE has not been investigated previously. Therefore, the primary objectives of this study were to determine: (1) movement patterns of *R. dominica* in wheat; and (2) if movement behavior is affected differently by the addition of the commercial DE products Dryacide[®], Insecto[™], and Protect-It[®] admixed with wheat.

2. Materials and methods

2.1. *Rhyzopertha dominica*

One- to 2-week old female *R. dominica* were selected from a pesticide-susceptible colony raised at ~30 °C and ~60% relative humidity (r.h.). The reason females were selected is that male *R. dominica* produce aggregation pheromones (Khorramshahi and Burkholder, 1981; Williams et al., 1981) which attract both sexes, therefore males were excluded to avoid bias in movement patterns caused by response to the pheromone. Voucher specimens from this colony were also deposited in the Kansas State University Museum of Entomological and Prairie Arthropod Research under Lot No. 162. *Rhyzopertha dominica* was sexed using the method described in Stemley and Wilbur (1966). Females were painted with one of three fluorescent pigment powders (DayGlo[®], Cleveland, OH, USA): Aurora Pink, ARC Yellow, or Saturn Yellow, which produced a pink, orange, and yellow color, respectively. Three different pigments were used to distinguish the beetles from each other. Each of the dry powder pigments (4 g) was mixed with 1 ml of Sun Light[®] concentrated dishwashing liquid, and 3 ml of deionized (DI) water. The dishwashing liquid acts as surfactant, which breaks the surface tension of the water to allow the dye particles to emulsify. This mixture created a quick-drying paint, which was applied to the elytra using the bent tip of a micro tool probe.

2.2. Experimental design

All tests were conducted at 32.2±1.7 °C in a single incubator (Forma Scientific, Marietta, OH, USA). Relative humidities of 57–60% were created in the incubator using a saturated solution of NaBr (Greenspan, 1997) in 1.9-L glass jar placed on the bottom shelf in the incubator. Mono-layers of wheat were created using two 40 × 20 cm double-paned glass plates held together with medium binder clips (5/8" capacity). Plexiglas spacers (approx-

mately 1.5 cm wide and 1.1 cm thick) placed between the plates around the side and bottom edges provided a gap of approximately 5 mm between the plates of glass, corresponding to approximately a mono-layer of wheat.

Two stands were created to hold four mono-layer plates at one time. These stands were manufactured from 1/2" (1.27 cm) square steel tubing, and were 42 cm tall, 62 cm long, with a support footing of 32 cm. A 20 × 1 cm lip of steel held the glass in place along with a small metal clip at the top of the stand. These stands held the mono-layer plates vertically and allowed observation of both sides. The mono-layer within the glass plates allowed for approximately 200 g of wheat.

Each run of the experiment consisted of an untreated control and three treatments of wheat admixed with three different commercial formulations of DE products at their labeled rates: Dryacide[®] 1000 ppm, Insecto[™] 500 ppm, and Protect-It[®] 400 ppm. These correspond to 0.20, 0.10, and 0.08 g of the DE product added to 200 g of wheat, respectively. The DE was weighed and admixed with wheat inside a glass jar, which was rotated and hand shaken for one minute to allow for adequate coating of the wheat. The plates were laid flat on a table and the wheat spread on what would be the back surface. The top plate was placed on top of the wheat and the two plates were clipped together. In the upright position the remaining wheat was carefully poured through the remaining space at the top of the mono-layer until the unit was filled to the top.

Each treatment in a run of the experiment contained three adult females, each with a different color painted on their elytra to allow for individual *R. dominica* to be traced through the mono-layer. Preliminary visual observations were made by simply observing movement of painted and unpainted beetles, and the paint did not appear to affect beetle movement in any way. The experiment was repeated 7 times for a total of 21 replicates; each individual was considered a replicate because the mono-layer was wide enough to accommodate three individual beetles. Pathways rarely crossed or intersected, and there was no apparent interference.

The females were dropped through the opening at the top of the glass plates and onto the top of the wheat at different locations. A UV light source was used at various observation times to provide light in the dark room, and beetle movement was marked as follows. Every 12 h for 5 d, the plates were examined by UV light, and the path left by the painted beetles was marked directly on the glass. This process took about 5–10 min total for the three beetles at each 12-h interval. After the last observation was made the beetles were removed from the wheat by sieving, tabulated as either dead or alive, and the glass plates were photocopied and the path tracing on the copy used to calculate movement parameters.

2.3. Data analysis

Paths were analyzed for each beetle (21 replicates) and average displacement per time interval, average

displacement for intervals with movement, and average turn angle were calculated for each interval. Using the initial line of movement as the main pathway, the protractor was adjusted against that line to determine the angle of deviation for the initial path. When no movement occurred during a step, data for that step was not used for calculating average turn. Results were analyzed using the General Linear Models (GLM) Procedure of the Statistical Analysis System (SAS Institute, 2002), with time as a repeated measure. Means for the movement data were transformed by square root and analyzed for differences using the Waller–Duncan k -ratio t -test of SAS. Table Curve (Systat Software, Point Redmond, CA, USA) was used to fit regressions to the distance *R. dominica* traveled during the exposure period.

3. Results and discussion

The distance *R. dominica* moved in each respective time interval during the 5 d was significant for the repeated measure time ($F = 28.5$; $df = 9, 180$; $P < 0.01$) but not treatment ($F = 2.6$; $df = 3, 600$; $P = 0.05$). A nonlinear regression model was fitted to the path length at each interval, and although the actual R^2 values were relatively low because of the variation in the data set, they comprised more than 96% of the maximum (MAX) R^2 (Fig. 1).

Total movement, average turn angle, and total number of turns were totaled for the 10 time periods. In all treatments, most of the movement occurred during the first three days of the test. The total distance moved by *R. dominica* during the total exposure period was shorter in the Dryacide[®] treatment compared to the untreated control, presumably because of the 100% mortality in the Dryacide[®] treatment. However, there was no difference among the DE treatments (Table 1). In addition, there was no significant difference among treatments with respect to the average turn angle ($F = 1.3$; $df = 3, 80$; $P = 0.28$), but *R. dominica* in the Dryacide[®] treatment turned less frequently compared to beetles in the untreated controls and the Protect-It[®] treatment ($F = 3.2$; $df = 3, 80$; $P = 0.03$) (Table 1), possibly because of the mortality in the Dryacide[®] treatment.

The movement of *R. dominica* in this study was typically downward, and appeared to slow during the 5-d test. The movement behavior of *R. dominica* is consistent with direct and indirect evidence from numerous studies (Sharangapani and Pingale, 1957; Surtees, 1965; Keever, 1983; Hagstrum et al., 1994; Vela-Coiffier et al., 1997; Hagstrum, 2001; Mohan and Fields, 2002), but our study is the first to directly observe movement behavior. Sharangapani and Pingale (1957) reported the movement of *R. dominica* as both downward and upwards. However, they used bags of grain in their study stacked one on top of the other, and denoted upward movement as movement from a release point below the bags as ‘up’, even though this was the only way for *R. dominica* to move into the food environment. Their results indicated that *R. dominica* was found nearest

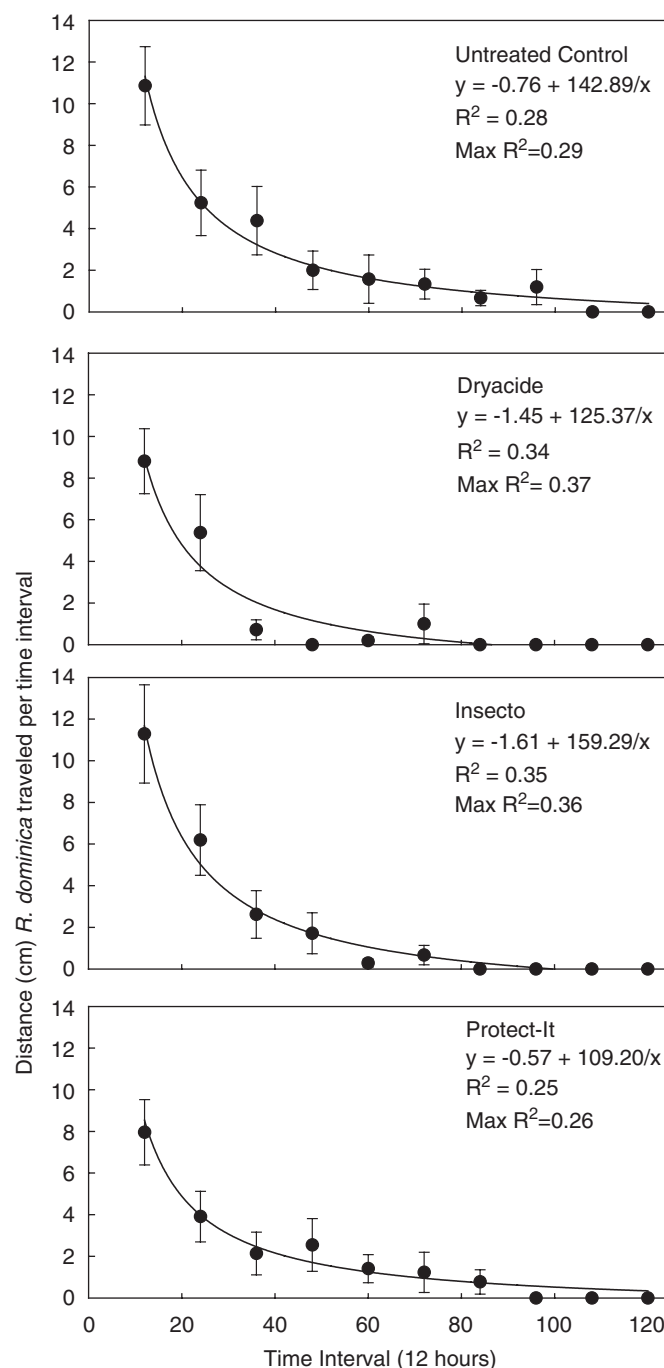


Fig. 1. Mean displacement of *R. dominica* at each 12-h time interval during the 5-d test period.

to the initial release point, and that their rate of movement is generally considered slow, which supports the findings in this current study as well.

Surtees (1964) conducted experiments on dispersion behavior of five species of stored-product insects, including *R. dominica*, using small boxes, each holding 25 kg of grain, with four layers separated by netting to allow for movement. Adults were released at the center of the upper layer on the grain. After “a prescribed period”, the box was broken down and the insect’s location within the box was

Table 1

The percentage mortality, total distance traveled, turning angles, and number of turns for *R. dominica* in response to various treatments with diatomaceous earth (mean \pm SEM)^a

Treatment	Mortality	Total distance	Average turn angle (°)	Number of turns
Untreated control	14.3 \pm 7.8 c	27.2 \pm 3.3 a	38.2 \pm 4.7 a	2.0 \pm 0.4 a
Dryacide [®]	100.0 \pm 0.0 a	15.7 \pm 2.5 b	33.0 \pm 7.4 a	0.9 \pm 0.1 b
Insecto TM	57.1 \pm 11.1 b	22.8 \pm 3.0 ab	55.2 \pm 12.0 a	1.5 \pm 0.2 ab
Protect-It [®]	71.4 \pm 10.1 ab	21.0 \pm 3.2 ab	47.0 \pm 8.3 a	1.9 \pm 0.3 a

^aMeans followed by different letters are significantly different ($P < 0.05$ Waller–Duncan k -ratio t -test, SAS Institute).

recorded. The experiment ran for 2 weeks. However, it was noted that most of the movement occurred within the first 48–72 h of the experiment. *Rhyzopertha dominica* was reportedly found more often in the lower layers of the boxes, although some lateral movement was noted. He also suggested that movement was gradual. In the current study, *R. dominica* moved quickly during the first 48–72 h, until eventually slowing or halting movement and in most cases remaining there for the duration of the study.

There was a significant treatment effect on *R. dominica* mortality ($F = 17.9$; $df = 3$; $P < 0.01$). The percentage mortality of *R. dominica* was significantly greater in all DE-treatments compared to the untreated control. Among DE treatments the highest mortality was observed in the Dryacide[®] treatment (Table 1). Results of this study indicate that movement of *R. dominica* was not decreased by the presence of the DE, even though mortality did relate directly to the labeled rate of the individual DE products. Because different products were tested, *R. dominica* mortality could have been affected by physical properties associated with the formulations (e.g., manufacturing techniques, additives, etc.) in addition to the concentration of DE. In addition, we did not show a clear repellent effect from the DE, as described by Mohan and Fields (2002) for *R. dominica* and other insect species. Stored-product insects can be repelled by DE applied to bulk grain (White et al., 1966; Mohan and Fields, 2002), but we did not see dramatic differences in movement patterns between *R. dominica* in the untreated wheat versus the DE-treated wheat.

In the past, large amounts of DE have been required to adequately control stored-product pests, which often resulted in physical problems and mechanical damage to grain handling equipment (Korunic et al., 1996). Newer formulations of DE are now available and are more effective than some of the older products. Physical problems such as decreased flow rate of grain and increased bulk density can occur when entire grain masses are treated (Korunic et al., 1996, 1998). Using DE as a surface treatment could possibly minimize these disadvantages to grain and to equipment. However, surface applications of DE could potentially influence the rate of *R. dominica* movement either increasing it (reducing exposure time), or reducing it (thereby increasing exposure time). Any repellent effect would also decrease exposure time if *R.*

dominica is trying to escape the DE. In our tests of direct observations of movement of adult females, the rate of movement through DE-treated wheat was no different than through untreated wheat. Moreover, the treatment that was the most effective on *R. dominica*, Dryacide[®], had the highest amount of DE (1000 ppm). Therefore, additional studies in actual grain bins are needed to determine if surface treatments at this rate are acceptable, and if using products with lower amounts of DE would provide adequate control of the pest population.

Acknowledgments

We thank Ralph Charlton, Kansas State University, and Steven Kells, University of Minnesota, for reviewing the manuscript prior to journal submission. We also thank Robert Rousser at the USDA Grain Marketing and Production Research Center (Manhattan, KS) for building the stands that held the mono-layer plates. We also thank J.M. Vardeman and Y. Chanbang for technical support. This research was supported in part by USDA-CSREES-RAMP Agreement no. 00-51101-9674. This is Contribution no. 05-298-J from the Kansas Agricultural Experiment Station.

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